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Therapeutic Approach to Correct Hematopoiesis and Anemia in Calves with Nonspecific Bronchopneumonia

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The paper describes an effective method to correct hematopoiesis and treat anemia in calves suffering from nonspecific bronchopneumonia. The method involves introducing the Vitamin Plus pine needle feed mix in a dose of 5-20 ml per head once daily for four weeks, starting at 30 days of age. This supplementation increases hemoglobin, hematocrit, the average concentration of hemoglobin in red blood cell and serum iron, while also boosting white blood cell, lymphocyte, and monocyte counts. The results show that adding Vitamin Plus to the diet positively influences anemia correction and improves immune response in calves. This method offers a natural and non-invasive alternative to conventional iron supplements and antibiotic treatments, which often carry risks of side effects and antimicrobial resistance. Implementing this dietary strategy in livestock management could reduce mortality rates, enhance growth performance, and improve farm profitability, making it a valuable addition to modern veterinary practice.

Keywords: Iron deficiency anemia, vitamin plus pine needle feed mix, hemosiderosis, iron dextran, gastrointestinal microflora

INTRODUCTION

The Vitamin Plus treatment, supplemented with pine needle feed, is a new and more integrated method of treating calves with nonspecific bronchopneumonia and iron deficiency anemia compared to the traditional method. Traditional methods primarily rely on the administration of iron-containing supplements, such as iron dextran, which is commonly used as ferrous sulfate and ferrous gluconate to treat iron deficiency anemia. While effective, these supplements are often associated with side effects, such as hemosiderosis and stress in animals, particularly in cases where the animals are already under physical strain due to disease. Studies have shown that iron dextran injections increased Fe concentrations but reduced hematocrit (Ht) level, red blood cell (RBC) count, and hemoglobin (Hb) level (Al-Anbakey *et al.*, 2024; Sickinger *et al.*, 2024). In addition, conventional therapies of nonspecific bronchopneumonia, which consist mainly of antibiotics, are likely to cause microbial resistance, toxic effects, and disruption of gastrointestinal microflora. These factors emphasize the need for alternative non-invasive modalities such as Vitamin Plus (Ferroni *et al.*, 2022). Previous studies have highlighted the effectiveness of pine needle extract supplementation and treatment in various animals. Chang *et al.* (2024) reported that pine needle supplementation promoted growth performance in broilers. They also recorded its gut microbiota modulating ability as they observed an increase in the amount of Rikenella, a beneficial bacterium in the rumen of cattle, when pine needle was temporarily introduced as an additive. Previous research has shown that pine needles can act as potent chelating agents for iron and exhibit antioxidant and antimicrobial activities (Hwang *et al.*, 2023; Rubens *et al.*, 2023). Ya-Xin *et al.* (2023) also displayed the effectiveness of pine needle extract as a substrate for rumen fermentation. They observed a 15% reduction in concentration of ammonia nitrogen, volatile fatty acids, gas production, and dry matter degradation when pine needle was used. Further studies by Han *et al.* (2022) exposed the potential of pine needle extract in countering respiratory diseases. Han *et al.* (2022) concluded that pine needle extracts reduced the hemagglutinin titer, inhibited H9N2 avian influenza virus nucleocapsid protein expression, and indirectly regulated type I and II interferon expression. The

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benefits of the Vitamin Plus treatment extend beyond the correction of iron deficiency anemia to encompass its broader therapeutic effects. It is a safer, more effective, and non-invasive solution for managing iron deficiency anemia and nonspecific bronchopneumonia in calves and a more natural option than synthetic options, hence lessening the risk of harmful side effects. Respiratory diseases in calves remain an urgent problem of veterinary medicine (Kamalieva *et al.*, 2020). The death rate and forced slaughter of calves from respiratory pathology can be 8.5-10.6 and 2.6-3.4%, respectively. In some farms, their death in combination with forced slaughter can reach 40-60%. According to most veterinarians, the decisive factor in nonspecific bronchopneumonia is a decrease in the resistance to adverse environmental influences due to disruptions in the technology of keeping and feeding animals (Nikulina *et al*., 2017**;** Ahmed and Abdullah, 2022). When growing young cattle, strict temperature requirements are imposed (Mecaj *et al*., 2023). Moderately low temperatures contribute to better use of feed and cold training of the animal body (Nasiyev *et al*., 2023). With significant temperature drops, calves develop signs of cold-related stress, which leads to growth failure, frequent gastrointestinal diseases, and bronchopneumonia. A lack of minerals in diets leads to rickets, white muscle disease, and anemia (Kurdeko *et al*., 2017). In Russian animal husbandry, when calves are raised in cold pens, iron deficiency anemia is most often registered. In addition to direct loss from animal deaths, large funds are spent on its correction and prevention (Zavalishina *et al*., 2011; Abutalip *et al*., 2024; Reshetnikova *et al*., 2024). Signs of anemia are detected in 6.9-29.3% of 1-3-day-old calves and about 20% of 30-40-day-old calves (Anokhin *et al*., 2003). With iron deficiency anemia, the qualitative and quantitative red blood cell (RBC) composition changes (Berata *et al*., 2024). The degree of so-called dyserythropoiesis depends on the severity of the decrease in plasma iron concentration (Idelson, 1981).

Iron-containing preparations are used to correct iron deficiency. The most effective iron dextran products are obtained by combining iron with the polysaccharide dextran, which easily forms colloidal solutions (Japaridze *et al.*, 2020). These include Impoferon, Imposil 200, Myofer, Armidextran, Ferrobal, Ferrodextran, Ferrodex, and Ferroglucine. Iron absorption begins 3-4 hours after administration of the preparation, is completed within 3-4 days (60% of the preparation), and is consumed by the body for about 30 days (Karput and Nikoladze, 2003). The disadvantage of these preparations is a pronounced side effect, which can lead to hemosiderosis and induce stress in some animals. Besides, these preparations are contraindicated for vitamin E deficiency.

The main method of treatment of nonspecific bronchopneumonia in calves is the use of antibacterial preparations. Irrational use of antimicrobial agents may be accompanied by toxic reactions, suppression of immunity, and disorders of vitamin metabolism, mainly associated with the suppression of the normal microflora of the gastrointestinal tract (Rahim *et al.*, 2020; Manaf *et al.*, 2023). Therefore, for pathogenetic and symptomatic treatment of animals with bronchopneumonia, it is recommended to use non-specific therapy preparations containing vitamins, trace elements, and biologically active substances with expectorant and anti-inflammatory effects (Baymenov *et al*., 2023). Saponins enhance the secretion of bronchial glands and dilute sputum. Essential oils have expectorant and antiseptic effects. More than 35 medicinal plants can be used to treat bronchopneumonia in animals. These plants do not have side effects even with prolonged use and are better tolerated; their effect is higher than from chemicals (Nikulina *et al*., 2017). Data were obtained on the positive effect of a feed additive containing 30% pine needle extract and 70% glycerin in calves from 28-30 days of age at a dose of 5.0-15.0 ml per head per day for 42 days, which helped eliminate diarrheal dyspeptic syndrome in 90% of cases and signs of bronchopneumonia in 100% of cases and increased serum iron concentration (Skornyakova *et al*., 2023). Under the conditions of production experiments on sucking calves, the effectiveness of a pine needle phytogenic immunomodulator for the correction of iron deficiency anemia in combination with the dyspeptic syndrome and eimeriosis was proven (Korotkii *et al*., 2023). A distinctive feature of Vitamin Plus is that it contains extracts of pine needles, the bark of aspen, poplar, and bird cherry, and vitamins A, D3, and E. Pine needles are rich in vitamin A, beta-carotene, B vitamins, vitamin H, K, PP, iron, cobalt, manganese, and zinc (Lapshin *et al.*, 2023). The protein composition of pine needles includes 19 amino acids (Levin and Repyakh, 1984). The bark of the black poplar contains tannins (3-9%), glycosides, salicin, alkaloids, flavonoids, and higher hydrocarbons (Yarulina, 2020). The bark of the European bird cherry contains tannins and organic acids (Khazipov *et al*., 2012). Vitamin A participates in oxidative processes, phosphoric, carbon, and lipid metabolism and increases the growth and development of young farm animals. Vitamin D3 is involved in the regulation of energy and mineral metabolism. Vitamin E participates in the regulation of carbohydrate and fat metabolism and increases the appetite and growth of animals (Baidalina *et al.*, 2024). Thus, the purpose of our study was to evaluate the effectiveness of a method for correcting hematopoiesis, in particular, iron deficiency anemia in calves with signs of nonspecific bronchopneumonia when using the Vitamin Plus pine needle feed mix in the diet of 30-day-old calves.

MATERIALS AND METHODS

***Study location and duration*:** The study was conducted at a dairy farm in the Kirov region (Russia) in the spring of 2024. The duration of the experiment was 28 days.

***Animals and housing conditions*:** The object of the study was the breeding heifers of the black-and-white Holstein breed grown in an unheated calf village up to 2.5 months of age. In the experiment, two groups (5 each) of 30-days old calves were formed with signs of nonspecific bronchopneumonia in mild and moderate degrees. The clinical picture in the experimental calves was characterized by slight depression, serous discharge from the nasal passages, an increase in body temperature by 0.5-1.0°C, and a rare, sometimes paroxysmal cough. During chest auscultation, harsh vesicular respiration was detected. Calves were housed in individual outdoor hutches, designed to provide adequate ventilation while protecting them from extreme weather conditions. These hutches were placed on straw bedding, which was replaced as needed to maintain hygiene and prevent bacterial infections. The calves were kept under natural daylight conditions, with no artificial lighting used. Calves were monitored daily for any signs of illness, including body temperature, respiratory symptoms, and feeding behavior.

***Diet and feeding regimen*:** All calves received a standardized diet formulated to meet their nutritional requirements for growth and immune support. The diet included:

- Whole milk (4–6 liters per day, divided into two feedings).
- Starter grain concentrate (18% crude protein, composed of crushed barley, wheat bran, soybean meal, and mineral-vitamin premix).
- Fresh water provided ad libitum.
- High-quality grass hay offered daily.

The experimental group received an additional Vitamin Plus supplementation mixed with their milk or warm water. The control group received only the standard diet, without any additional supplements.

***Experimental design*:** The control group of calves (n=5) received the basic diet according to the recommended zootechnical standards, and the experimental group (n=5) received the basic diet and Vitamin Plus at the rate of 5-20 ml per head, once a day with combined milk or warm water for a 4-week course without interruptions. The supplement was dozed gradually, increasing the dose by 5 ml every 7 days. During the entire experimental period, the animals were monitored and examined twice for a total blood count (TBC), white blood cell (WBC) profile, and serum iron concentration.

***Clinical and laboratory evaluations*:** The TBC and leukoformula parameters were determined using a HumaCount 30TS hematological analyzer, and the concentration of iron in blood serum was determined using an iMage-V7 biochemical analyzer.

***Data processing*:** The average standard deviation (ASD) for the group was calculated using Microsoft Excel 2010 and Student's t-test.

RESULTS

In the study of the TBC, signs of hematopoiesis disorders (anemia syndrome) and the inflammatory process were observed (Tables 1-3). In the calves of the control and experimental groups with bronchopneumonia, there was a decrease in the level of hemoglobin (HGB) and hematocrit (HCT) in the blood. As for RBCs, their number was within the upper limit of the norm and ranged from 9.77±0.69 to

Table 1. Indicators of the TBC before and after the use of Vitamin Plus, M±m.

Indicator	Reference values	Control group, n=5		Experimental group, n=5	
		before	after 28 days	before	after 28 days
RBCx10^{12}/l	5.0-10.0	10.72±1.60	9.35±0.37	9.77±0.69	9.84±0.69
HGB, g/l	90-120	103.40±6.91	97.20±1.09	102.60±1.67	108.80±1.30*
HCT, %	28-46	29.57±2.42	26.83±0.55	29.35±0.25	30.27±0.32*
MCV, fL	37-51	29.52±1.60	27.50±1.64	30.06±1.41	30.86±2.09
MCH, pg	13-18	10.42±0.47	10.36±0.22	10.50±0.22	10.84±0.57
MCHC, g/l	330-370	353.80±2.92	354.20±2.77	351.40±2.10	357.80±1.53*
PLT, x10^{9}/l	260-700	517.00±51.7	184.40±60.9	366.20±41.3	337.00±94.0
Procalcitonin (PCT), %	0.068-0.176	0.30±0.06	0.09±0.04	0.19±0.11	0.16±0.05
MPV, fL	4.5-6.7	4.68±0.15	5.30±0.55	4.76±0.50	4.94±0.48

Note: *the difference compared to the indicator before treatment is significant (P<0.05)

Table 2. Serum iron indicators before and after the use of Vitamin Plus, M±m.

Indicator	Reference values	Control group, n=5		Experimental group, n=5	
		before	after 28 days	before	after 28 days
Iron, mmol/l	10-30	9.2±1.21	10.4±0.83	10.02±0.51	11.58±0.42*

Note: *the difference compared to the indicator before treatment is significant (P<0.05)

Table 3. WBC profile of calves' blood before and after use of Vitamin Plus, M±m.

Indicator	Reference values	Control group, n=5		Experimental group, n=5	
		before	after 28 days	before	after 28 days
WBCx10^9/l	8.0-16.0	10.87±3.11	8.82±0.69	9.58±1.33	9.13±1.48
LYM, x10^9/l	2.5-7.5	2.33±1.11	2.55±0.63	2.97±0.88	3.15±0.82
MID, x10 ^{9}/l	0.0-0.84	0.22±0.31	0.27±0.44	0.16±0.09	0.27±0.44***
GRA, x10 ^{9}/l	0.6-6.7	6.34±1.10	6.10±0.59	6.13±0.59	5.50±0.95
Leukoformula:					
LYM, %	40-75	32.78±1.45	34.82±1.40	34.00±1.03	39.32±1.56*
MID, %	2-7	2.06±1.99	2.08±0.97	0.90±0.27	2.64±0.69*
GRA, %	25-51	65.14±2.25	60.36±1.34	65.08±1.60	58.02±2.61*

Note: the difference compared to the indicator before treatment is significant: *P<0.05; ***P<0.001

10.72±1.6 x 10^{12}/l. When analyzing the RBC morphometric characteristics in sick calves, a decrease in the mean corpuscular volume (MCV) of RBCs and the mean corpuscular HGB (MCH) in one RBC was noted compared with the norm. These changes indicate the development of microcytic hypochromic anemia. After the calves received Vitamin Plus, the HGB level in the blood of experimental calves significantly increased by 6% (P<0.05) and was 11.9% higher than in the control group. HCT also significantly increased by 3.1% (P<0.05) compared with the beginning of the experiment and by 12.8% compared to the control group. As for the RBC indices, the MCH and MCV values remained approximately at the same level, and the MCH concentration (MCHC) value significantly increased by 1.8% (P<0.05) and was 1% higher than in the control group. The dynamics of platelets (PLT) showed a significant decrease in their number in the control group (Table 1). To confirm iron deficiency anemia, the serum iron content was analyzed, which was reduced in the control and experimental groups of animals and ranged from 9.2±1.21 to 10.02±0.51 mmol/l. After the calves received Vitamin Plus, the level of iron in the blood serum of experimental calves significantly increased by 15.65% (P<0.05) and was 11.3% higher than in the control animals (Table 2). When analyzing the WBC profile of the blood of 30-day-old calves with bronchopneumonia, we found that the WBC number was within the reference values (Table 3). When studying the WBC type, we noted that the number of lymphocytes (LYM) in the peripheral blood of calves was low (lymphopenia) and ranged from 2.33±1.11 to 2.97±0.88 x 10^9/l. The number of granulocytes (GRA) was increased (neutrophilia) and amounted to 6.34±1.1-6.13±1.11 x 10^9/l. The WBC formula of all calves also showed a very high concentration of GRA neutrophils (65.08-65.14%) and a low LYM concentration (32.78-34%). These changes characterize the inflammatory process in the lungs and low resistance. After the calves received Vitamin Plus, the level of WBC in the peripheral blood of the experimental calves remained at the same level, unlike the control group, where there was a decrease in their number by 18.9%. The dynamics of various WBC types in the blood of experimental calves were characterized by an increase in the LYM number by 6.1% and monocytes by 1.7 times (P<0.001), while the number of neutrophil GRA decreased by 10.3%. In the WBC formula of the experimental calves, the LYM content significantly increased by 5.3% (P<0.05) and monocyte content by 1.7% (P<0.05), while the neutrophil content decreased by 6.9% (P<0.05). As for the control calves, the ratio of LYM, monocytes, and neutrophils remained approximately at the same level. The changes occurring in the WBC profile of the blood of experimental calves characterize the strengthening of the immune response and recovery.

DISCUSSION

The results of this study demonstrate that the inclusion of Vitamin Plus, a pine needle feed mix, in the diet of calves with nonspecific bronchopneumonia significantly improves hematopoiesis and corrects anemia. Specifically, the study found that Vitamin Plus increased hemoglobin (HGB), hematocrit (HCT), and serum iron levels, while also positively affecting white blood cell (WBC) profiles, particularly increasing lymphocyte (LYM) and monocyte counts. The study by Kuzmina *et al.* (2020) also reported similar results. In their study on the effect of pine needle extract as a feed additive on the young cattle's productivity, they observed an increase in blood count and hematological activities. Although most studies administer pine needle extract for a short period of time, the current study extends these findings by specifically quantifying the improvements in hematological parameters, such as HGB, HCT, and WBC profiles, over a 4-week period. This provides a more detailed understanding of the timeline and extent of the benefits conferred by Vitamin Plus. Our findings also agree with the study conducted by Tereshchenko *et al.* (2020) who observed similar results such as increased RBC, WBC, and a stable PCT level. The study also aligns with the findings of Wheat *et al.* (2020) and Belewu *et al.* (2024) on the basis of exploring non-specific therapy. The present study shows that Vitamin Plus aids in hematopoiesis in addition to stimulating immune responses, as indicated by the rise in lymphocyte and monocyte counts. This would imply that Vitamin Plus can serve as a more holistic approach to the management of

bronchopneumonia in calves, effectively tackling the hematological and immunological component of the disease. Our study diverges from previous research, such as Chang *et al.* (2024), in the areas of dosage and administration, suggesting that the optimal dosage and duration of treatment may vary depending on the specific condition and causative factors. Further research is recommended to determine an effective treatment regime for different health issues in calves (Sherimova *et al.*, 2022). The economic feasibility of using Vitamin Plus in calf-rearing practices is promising, particularly given the high costs associated with treating respiratory diseases and anemia in calves. Studies have shown that vitamin blends as supplements can reduce feeding costs since they can be organically obtained (Silva *et al.*, 2022). Additionally, the improved health and growth rates of calves could lead to higher productivity and reduced mortality rates, further enhancing the economic benefits. The economic feasibility of vitamin plus treatment is also limited by the availability of pine needle, and further research is recommended for phytogenic alternatives like grape extract, especially in places where pine needle is not accessible (Molosse *et al.*, 2023). A macro-level analysis suggests that the use of a natural, phytogenic feed additive will appeal to consumers and farmers who are increasingly concerned about the use of antibiotics and synthetic drugs in livestock production, potentially opening up new market opportunities for producers. In conclusion, the application of Vitamin Plus in calf rearing is economically viable, providing an affordable means of enhancing calf productivity and health and possibly minimizing the use of more costly and less environmentally sound treatments (Mukhamadiyev *et al.*, 2023; Hilmiati *et al.*, 2024). Additional economic evaluation, such as cost-benefit analysis in relation to conventional treatments, would be valuable to determine the full financial impact of adopting the use of Vitamin Plus in commercial calf-rearing enterprises (Islami *et al.*, 2024; Suarsa *et al.*, 2024).

***Conclusion*:** Our studies showed that the use of Vitamin Plus in the diet of calves in the second month of life has a positive effect on the correction of hematopoiesis, particularly anemic syndrome. This method can be recommended for use in the treatment of nonspecific bronchopneumonia in calves. While this study had a relatively small sample size, the observed improvements in hematopoiesis and immune function indicate that Vitamin Plus has significant potential as a natural supplement for calves with nonspecific bronchopneumonia. Although the short duration limited the ability to assess long-term effects, the results demonstrated a clear positive impact on hemoglobin levels, hematocrit, and white blood cell profiles. Future research should build on these promising findings by conducting larger-scale studies, extending the observation period, analyzing the contributions of individual Vitamin Plus components, and comparing its efficacy with standard antibiotic treatments while also assessing economic feasibility and animal welfare outcomes.

***Authors' contribution statement*:** Conceptualization, V.K. and A.R.; Methodology, O.S.; Validation, V.R., D.M. and V.K.; Data Curation, D.M.; Writing – Original Draft Preparation, O.S.; Writing – Review & Editing, A.R.; Supervision, V.K.; Project Administration, D.M.

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***Ethical statement*:** All procedures involving animals were carried out in accordance with ethical guidelines for the care and use of animals in research.

***Availability of data and material*:** The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

***Informed consent*:** N/A

***Consent for publication*:** All authors have read and approved the final version of the manuscript and consent to its publication.

***SDGs addressed*:** Zero Hunger, Good Health and Well-being.

***Policy referred*:** The study advocates for a shift in livestock health management policy towards the use of natural, phytogenic feed additives—like the Vitamin Plus pine needle mix—as a safer, more sustainable alternative to conventional iron supplements and antibiotics.

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